The Effects of Temperature and Humidity on Longevity of Insecticide Resistant and Susceptible *Musca domestica* Linnaeus[1]

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Abstract: The effects of three humidities and three temperatures were tested on the longevity of both sexes of two strains of *Musca domestica* L. House flies survive longest at the combination of the lowest temperature (15°C) and the highest humidity tested (97%). Their longevity is least at the highest temperature (35°C) and lowest humidity (7%) combination, and the intermediate temperature-humidity combinations are intermediate in effect on longevity with the effect of temperature in all cases greater than the effect of humidity. There is no difference in longevity between the insecticide resistant and susceptible strains tested. The factor of desiccation appears to be the primary mechanism involved in determining longevity with temperature determining the rate.

It is believed that temperature and relative humidity are of great importance in determining the times and places of insect distribution. Furthermore the relationship of some fly-borne diseases to climatic conditions has been known for some time, but in the field it is difficult to isolate the factors of temperature and humidity from interactions with such factors as sunlight, predators, food supply, etc. which also probably serve to limit distribution. For this reason it was desirable to test the effects of these factors under controlled laboratory conditions where the effects of temperature and humidity could be isolated and their relative importance assessed in determining the longevity of house flies.

It was decided to compare males with females and to compare an insecticide susceptible strain with an insecticide resistant strain of house flies (*Musca domestica* L.) for response to different temperature and humidity combinations in an effort to determine if any differences between sexes or strains might exist and further to give some insight into the possible mechanisms involved in determining longevity at different temperature and humidity combinations. These

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tests were designed to approximate the range of temperature and humidity variations to which house flies are commonly exposed in nature.

MATERIALS AND METHODS

Musca domestica of the Rutgers A-strain and the World Health Organization IN-strain were reared separately at an approximately constant temperature of 26°C. The flies were reared on CSMA artificial fly larval medium (Ralston Purina Co.) by the standard method as outlined in the Soap and Chemicals Specialties Blue Book (1967) and were fed reconstituted dry skim milk after emergence as adults. The flies were anesthetized with carbon dioxide, separated as to sex, and weighed prior to insertion into the humidity chambers.

The humidity chambers were clear plastic cylindrical containers with a tight-fitting plastic top and measured 15.2 cm inside diameter by 6.5 cm in height. Each chamber contained 100 ml of a saturated salt solution with an aluminum screen elevated 3 cm above the bottom of the dish separating the flies from the solution. The salts, NaOH, K_2CO_3, and K_2SO_4, were dissolved in distilled water until the solutions reached saturation, and then an excess of salt was added. According to Winston and Bates (1960) these solutions give constant humidities over a long period of time with relative humidities of 7% for NaOH, 43% for K_2CO_3, and 97.5% for K_2SO_4 at 25°C. The humidity levels at 15°C and 35°C varied approximately 1.5% from the values at 25°C. Each humidity chamber had a small plastic dish with approximately 2 gm of powdered dry milk for food.

Twenty house flies were inserted into the chambers through a small hole in the top by means of a glass tube open at both ends. The anesthetized flies were placed in the tube, counted, and then dropped into the humidity chambers which were kept in incubators. The three incubators used were maintained at 15°, 25°, and 35°C with the light constant at approximately 70 footcandles. Twelve humidity chambers were placed in each incubator with four at each of the three humidities. Of the four chambers at the same humidity, two were used for males and two for females. Thus, there were three incubators with four chambers at each of the three humidities in each incubator with 20 flies in each chamber resulting in a total of 720 flies for each test.

Tests were run over weekends, beginning on Friday at 9:00 A.M. and generally ending on the following Tuesday evening when all the flies had died. The flies were observed and the number dead was recorded every four hours with the exception of the 5:00 A.M. reading. Only one strain was tested at a time and a total of six tests were run in pairs alternately between strains on successive weekends with three tests of each strain. After each pair of tests the humidity chambers were rotated among the incubators. Because of the limited shelf size in the incubators, it was necessary to place one group of four chambers on a lower shelf where they were exposed to somewhat reduced light, but this

Table 1. Comparison of LT_{50} values for the 2 strains of flies (average of 3 tests).

	LT_{50} values expressed in hours					
	A (7% humidity)		C (43% humidity)		E (97% humidity)	
	males	females	males	females	males	females
A-strain						
15°C	39	43	49	53	57	58
25°C	17	18	23	22	23	20
35°C	7.6	7.2	9.6	9.2	10.0	9.6
IN-strain						
15°C	31	54	42	59	53	70
25°C	14	21	20	27	23	26
35°C	6.0	7.6	6.6	9.2	9.6	10.4

was compensated for by rotating the containers after each pair of tests so that each of the three humidities was placed on the lower shelf for one pair of tests. To compensate for any temperature gradient in the incubators the chambers were randomly distributed on the shelves.

Results and Discussion

The data were graphed for each temperature-humidity combination separately for each strain, (Figs. 1 and 2) but combining the sexes, the replicates, and the three tests so that each curve represents 240 flies. For direct comparisons of the series of curves, LT_{50} determinations were made for each temperature-humidity combination. The LT_{50} is the time of exposure when 50% of the flies were dead and was determined (Table 1) from similar graphs where data for the sexes were plotted separately.

The LT_{50} values in Table 1 were statistically analyzed as a $3 \times 3 \times 2 \times 2$ factorial experiment to determine the significance of the differences between temperatures and humidities and to determine if there was any difference between the IN and A strains. The difference between both humidities and temperatures was highly significant at the 95% level of significance and the interaction between temperature and humidity effects was significant as well. The fact that the humidity effect is not the same at different temperatures can be seen on the graphs as the generally increased separation of the lines with decreasing temperatures, resulting from the increased longevity of flies at lower temperatures.

The effects of temperature on longevity were greater than the humidity effects and clearly indicated an increased longevity with decreased temperature. The differences between strains were determined not significant as the two strains responded similarly to all treatments. It was found that the higher hu-

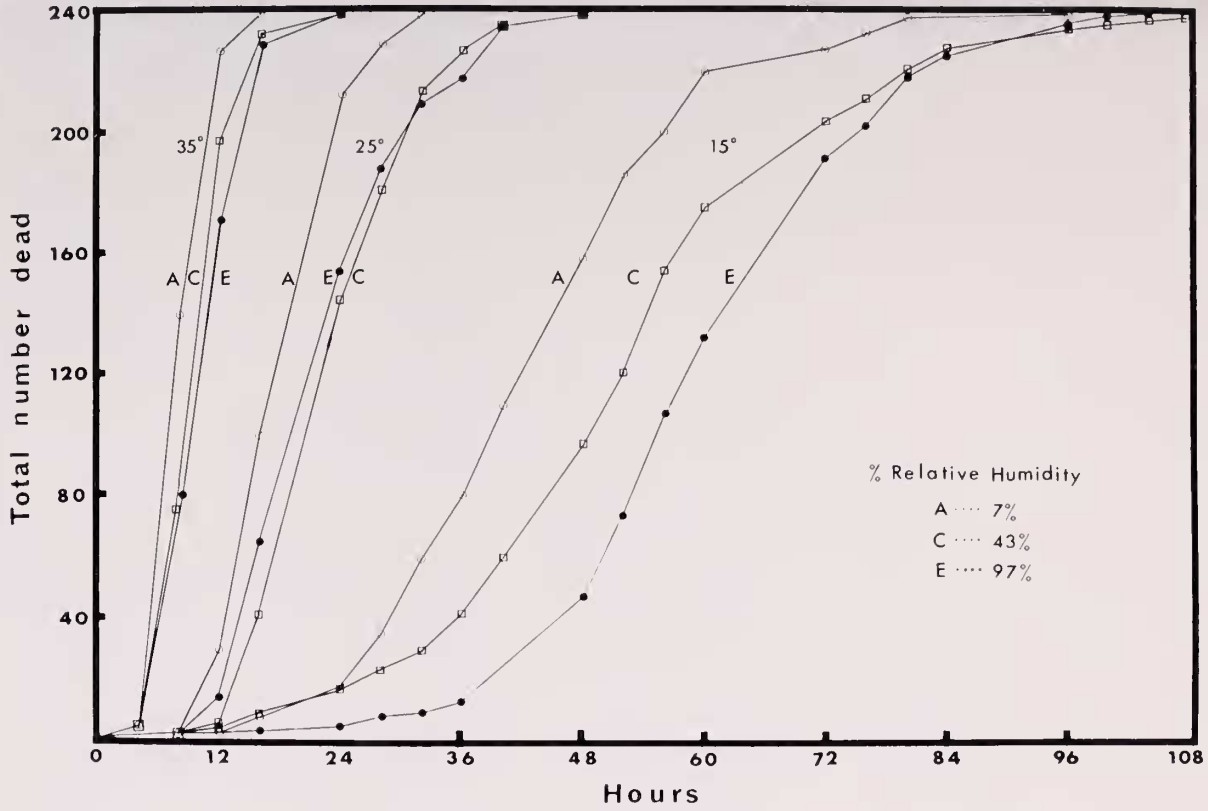

FIG. 1. Rutgers A-strain flies showing the effects of the 3 temperatures and the 3 humidities on longevity.

Table 2. Weight and longevity values for sexes and strains (average of 3 tests).

	A-strain	IN-strain
Avg. wt. males	43.6 mg	39.7 mg
Avg. LT_{50} males	26.1 hr	22.8 hr
Avg. wt. females	47.8 mg	58.9 mg
Avg. LT_{50} females	26.7 hr	31.6 hr

midities resulted in progressively greater longevity at the three temperatures tested with the exception of a slight reversal of the intermediate and high humidity effects at 25°C for the A strain.

The survival differences between the sexes were found to vary greatly from test to test, but these differences could be directly correlated with the weight differences between the groups of flies. In cases where the sexes weighed about the same, they survived about the same. There was a greater weight difference between the sexes in the IN-strain than in the A-strain. This resulted in large survival differences between the sexes in the IN-strain, but small differences in the A-strain (Table 2).

Behavioral differences at the different temperatures were observed shortly after the flies were inserted into the humidity chambers. The flies exposed at 15°C moved very little, if at all, but the flies at 35°C were in constant motion, both crawling and flying, and the flies at 25°C were intermediate in activity. This behavioral pattern was apparent in all six tests for the duration of each test. It was suspected that the flies died from lack of water and that as a result of this water-loss they would decrease in weight during the test. To determine if this water-loss was the same at the three humidities, 20 flies were weighed and exposed to each of the three humidities without food for 10 hours and then anesthetised and weighed again. Those flies exposed at 97%, 43% and 7% humidity lost an average of 3.09 mg, 3.67 mg, and 4.62 mg per fly, respectively. These results indicate that there is a greater desiccation of the flies at lower humidities and serve, in part, to explain the differences in longevity at different humidity levels.

The flies were observed to be feeding on the dry powdered milk at frequent intervals during the tests and a surprising number of flies died in the milk dishes. At the high, intermediate and low humidity there was an average of 1.9, 3.1, and 5.1 flies, respectively found dead in the milk dish. By calculating the area of the milk dish and comparing it to the area of the humidity chamber, it was determined that by random chance an average of 1.4 flies should have died in each dish. The fact that in all cases more flies died in the powdered milk than would be expected by chance alone probably results from the fact that the flies were feeding on the powdered milk, but it was suspected that

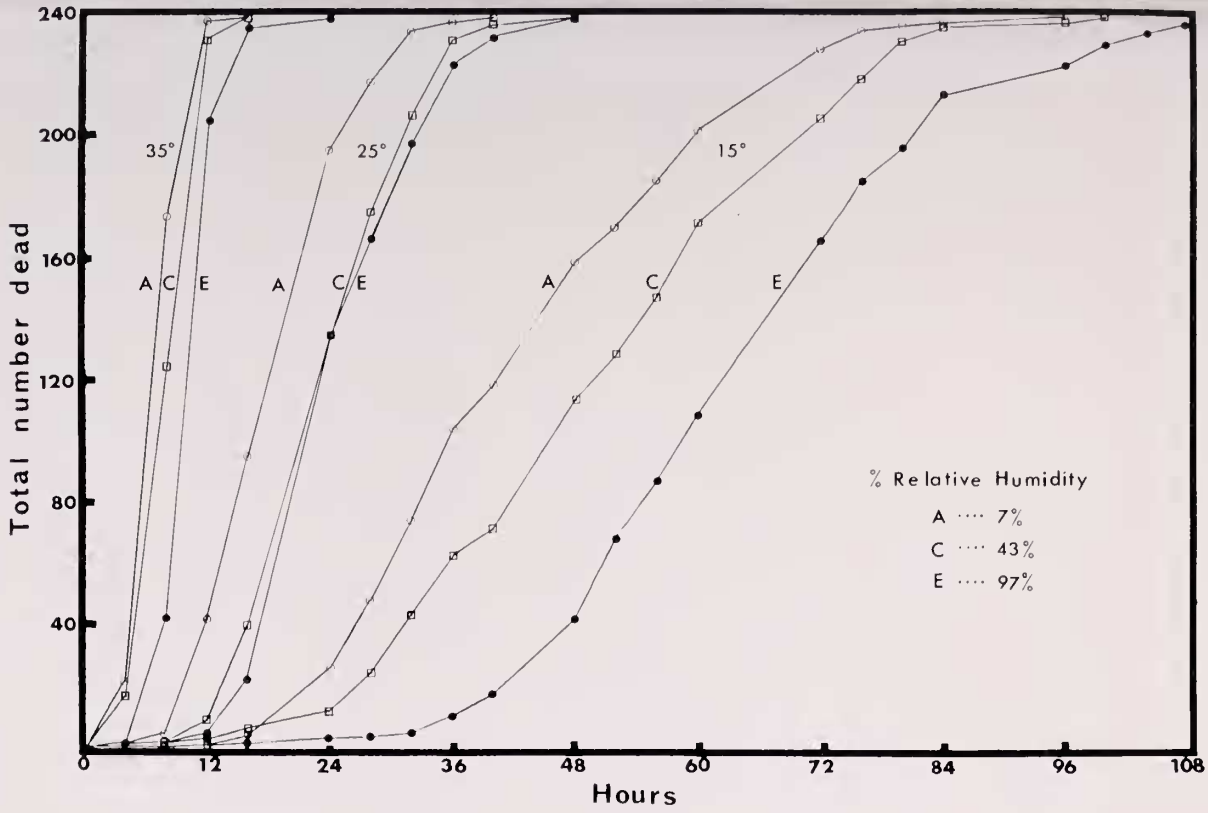

FIG. 2. World Health Organization IN-strain flies showing the effects of the 3 temperatures and the 3 humidities on longevity.

the differences between humidities might result from an ability of the flies to detect the small amount of moisture present in the powdered milk when they were suffering from desiccation. To test this hypothesis 12 dishes of powdered milk were weighed and placed in 12 humidity chambers with four at each of the three humidities. After 24 hours exposure these dishes were weighed again. At the lowest humidity the powdered milk lost an average of 0.7 mg per dish. At the intermediate humidity the powdered milk gained an average of 3.6 mg per dish, and at the highest humidity gained an average of 22.9 mg per dish during the 24 hour period. The results of this test indicate that at the lowest humidity the powdered milk had more moisture than the atmosphere and at the other two humidities there was more moisture in the air than in the milk. The flies were evidently able to detect these moisture differences and this resulted in the substantially different numbers of flies which died in the milk dishes at different humidities.

The results of these experiments differ somewhat from what was expected on the basis of the experiments of a similar nature found in the literature. Prevalent in the literature, Headlee (1917), Beattie (1928), is an idea that high humidity inhibits evaporative cooling and thus proves rapidly lethal at high temperatures (usually over 100°F). The results of this experiment indicate that the flies survive best at the high humidities at all temperatures though extreme temperatures were not studied. The desiccation of the flies seemed to be the primary mechanism involved in the death of the flies in this experiment. Even at high humidities the flies lost 6% weight in 10 hours. If inhibition of evaporative cooling played an important role in determining longevity, those exposed at the intermediate humidity and high temperature would be expected to survive better than those exposed to the high humidity and high temperature. This clearly is not the case.

It was expected that possibly the A-strain, as a side effect of selection for insecticide resistance might have developed a greater tolerance to a desiccation environment either as a result of a thickened cuticle or increased weight. Such clearly was not the case as the A-strain (resistant) averaged 45.7 mg and the IN-strain (susceptible) averaged 49.3 mg per fly and the difference in survival between the strains was extremely small and not statistically significant.

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